

**RESPONSE TO RESTRICTION REQUIREMENT**

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Serial No.: 10/539,765

Dkt: 1662.004US2

Filed: February 8, 2006

Title: METHODS AND COMPOSITIONS FOR SELECTIVELY ENRICHING MICROBES

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**IN THE CLAIMS**

Please amend the claims as follows.

1. (Original) A method comprising: incubating a first sample suspected of containing one or more competitor microbes and one or more target microbes in an acidic medium to produce a second sample; wherein incubating the first sample in the acidic medium can generate a second sample that has a higher percentage of non-pathogenic or pathogenic *Escherichia coli* than the first sample.
2. (Original) The method of claim 1, wherein the pathogenic *Escherichia coli* is enterohemorrhagic *Escherichia coli*, enteropathogenic *Escherichia coli*, or enterotoxigenic *Escherichia coli*.
3. (Original) The method of claim 1, wherein one or more competitor microbes is a competitor bacterium.
4. (Original) The method of claim 3, wherein one or more competitor bacteria are killed or growth inhibited during incubation in the acidic medium.
5. (Original) The method of claim 1, wherein one or more target microbes is selected from the group consisting of *Salmonella*, *Shigella*, *Staphylococcus*, *Klebsiella*, *Escherichia*, *Listeria*, *Morganella*, *Enterobacter*, *Serratia*, *Yersinia*, *Bacillus* and *Hafnia*.
6. (Original) The method of claim 1, wherein one or more target microbes is a pathogenic bacterium.

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7. (Original) The method of claim 6, wherein the pathogenic bacterium is enterohemorrhagic *Escherichia coli*, enteropathogenic *Escherichia coli*, or enterotoxigenic *Escherichia coli*.
8. (Original) The method of claim 6, wherein the pathogenic bacterium is *Escherichia coli* O157:H7.
9. (Original) The method of claim 1, wherein the pH of the acidic medium is between 1 and 6.
10. (Original) The method of claim 1, wherein the pH of the acidic medium is between 2 and 4.
11. (Original) The method of claim 1, wherein the pH of the acidic medium is between 2 and 3.
12. (Original) The method of claim 1, wherein the first sample is an environmental sample.
13. (Original) The method of claim 1, wherein the first sample is a water sample.
14. (Original) The method of claim 1, wherein the first sample is a food sample.
15. (Original) The method of claim 1, wherein the first sample is a bodily sample.
16. (Original) The method of claim 1, wherein the acidic medium is selected from the group consisting of GYT medium, LB medium, M9 minimal medium, NZCYM medium, NZYM medium, SOB medium, SOC medium, TB medium, 2x YT medium, BHI, and TSB.

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17. (Original) The method of claim 1, wherein the acidic medium comprises a selective agent.
18. (Original) The method of claim 17, wherein the selective agent is an antibiotic.
19. (Original) The method of claim 17, wherein the selective agent is a bacteriophage.
20. (Original) The method of claim 17, wherein the selective agent is a nutritional supplement.
21. (Original) The method of claim 17, wherein the selective agent is an inorganic selective agent.
22. (Original) The method of claim 17, wherein the selective agent is an organic selective agent.
23. (Original) The method of claim 17, wherein the selective agent is tellurite, selenite or sorbitol.
24. (Original) The method of claim 1, wherein the first sample is incubated in the acidic medium for 0.1 to 10 hours.
25. (Original) The method of claim 1, wherein the first sample is incubated in the acidic medium for 1 to 4 hours.
26. (**Currently Amended**) The method of claim 1, wherein the first sample is incubated in the acidic medium for 1 to 2.5 hours a length of time that causes

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death of or damage to the competitive microbes when they are later incubated in a growth medium.

27. (Original) The method of claim 1, wherein the first sample is incubated in the acidic medium at a temperature that is about 5°C to about 20°C.
28. (**Currently Amended**) The method of claim 1, wherein the first sample is incubated in the acidic medium at a temperature that is about 20°C to ~~about 45°C~~  
5°C to about 35°C.
29. (Original) The method of claim 1, wherein the first sample is incubated in the acidic medium at a temperature that is about 20 °C to 22 °C.
30. (Original) The method of claim 1, wherein the first sample is incubated in the acidic medium at a temperature that is between 37°C and 70°C.
31. (Original) The method of claim 1, wherein the acidic medium comprises glutamate.
32. (Original) The method of claim 1, further comprising detecting one or more target microbes in the second sample.
33. (Original) The method of claim 32, wherein one or more target microbes is detected using selective growth media.
34. (Original) The method of claim 33, wherein the selective growth media comprises an antibiotic.

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35. (Original) The method of claim 33, wherein the selective growth medium comprises a bacteriophage.
36. (Original) The method of claim 33, wherein the selective growth medium comprises a nutritional supplement.
37. (Original) The method of claim 33, wherein the selective agent is an inorganic selective agent.
38. (Original) The method of claim 33, wherein the selective agent is an organic selective agent.
39. (Original) The method of claim 33, wherein the selective growth medium comprises tellurite, selenite, or sorbitol.
40. (Original) The method of claim 33, wherein the selective growth medium comprises a medium selected from the group consisting of GYT medium, LB medium, M9 minimal medium, NZCYM medium, NZYM medium, SOB medium, SOC medium, TB medium, 2x YT medium, BHI, and TSB.
41. (Original) The method of claim 32, wherein one or more target microbes is detected using antibodies directed against the target microbe, enzyme-linked immunosorbent assay, or radioimmunoassay.
42. (Original) The method of claim 32, wherein one or more target microbes is detected using polymerase chain reaction.

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43. (Original) A kit comprising an acidic medium, a packaging material and instructions for using the acidic medium for enriching a sample with at least one target microbe.
44. (Original) The kit of claim 43, further comprising a growth medium.
45. (Original) The kit of claim 43, further comprising a pH modifier.
46. (Original) The kit of claim 43, further comprising a means to detect the target microbe.
47. (Original) The kit of claim 43, wherein the target microbe is a bacterium.
48. (Original) The kit of claim 47, wherein the bacterium is enterohemorrhagic *Escherichia coli*, enteropathogenic *Escherichia coli*, or enterotoxigenic *Escherichia coli*.
49. (Original) The kit of claim 47, wherein the bacterium is *Escherichia coli* O157:H7.
50. (Original) The kit of claim 47, wherein the bacterium is *Shigella*.
51. (Original) A kit comprising packaging material, an acidic medium, a growth medium, and a means for detecting bacteria.
52. (Original) The kit of claim 51, wherein the acidic medium and the growth medium are in liquid form.

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53. (Original) The kit of claim 51, wherein the acidic medium and the growth medium are in dry form.
54. (Original) The kit of claim 53, further comprising sterile water.
55. (Original) The kit of claim 51, further comprising a pH modifier.
56. (Original) A kit comprising packaging material, culture media, a first pH modifier, and a second pH modifier, wherein addition of the first pH modifier to the culture media produces an acidic medium and addition of the second pH modifier to the acidic medium produces a growth medium.
57. (Original) The kit of claim 56, wherein the first pH modifier is an organic acid.
58. (Original) The kit of claim 56, wherein the organic acid is selected from the group consisting of lactic acid, formic acid, acetic, propionic, and butyric.
59. (Original) The kit of claim 56, wherein the first pH modifier is an inorganic acid.
60. (Original) The kit of claim 56, wherein the first pH modifier is HCl, HF, HBr, H<sub>2</sub>SO<sub>4</sub>, or H<sub>3</sub>PO<sub>4</sub>.
61. (Original) The kit of claim 56, wherein the second pH modifier is NaOH or KOH.